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Assessing the bioavailability and bioaccessibility of metals and metalloids

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Abstract Bioavailability (BA) determines the potential harm of a contaminant that exerts on the receptor. However, environmental guidelines for site contamination assessment are often set assuming the contaminant is 100 % bioavailable. This conservative approach to assessing site risk may result in the unnecessary and expensive remediation of a contaminated site. The National Environmental Protection Measures in Australia has undergone a statutory 5-year review that recommended that contaminant bioavailability and bioaccessibility (BAC) measures be adopted as part of the contaminated site risk assessment process by the National Environment Protection Council. We undertook a critical review of the current bioavailability and bioaccessibility approaches, methods and their respective limitations. The 'gold' standard to estimate the portion of a contaminant that reaches the system circulatory system (BA) of its receptor is to determine BA in an in vivo system. Various animal models have been utilised for this purpose. Because of animal ethics issues, and the expenses associated with performing in vivo studies, several in vitro methods have been developed to determine BAC as a surrogate model for the estimation of BA. However, few in vitro BAC studies have been calibrated against a reliable animal model, such as immature swine. In this review, we have identified suitable methods for assessing arsenic and lead BAC and proposed a decision tree for the determination of contaminant bioavailability and bioaccessibility for health risk assessment.

Keywords Bioavailability · Bioaccessibility · Metals · Metalloids · Risk assessment · Site contamination

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Background, aim and scope

In Australia, the National Environmental Protection Measure (NEPM) conducted a statutory 5-year review resulting in a Review Report consented to by the National Environment Protection Council (NEPC) in November 2006. One of the recommendations was related to the provision of a guidance document on bioavailability and associated bioaccessibility testing methods and their application. During the implementation phase of this recommendation, we were engaged, in 2010, to conduct a literature review and explore the possibility for the production of a national guidance document that can be considered for inclusion in future NEPM documents.

The current NEPM has been in place for over a decade (NEPC 1999). The document provides guidance for site contamination assessment and national health investigation levels (HIL) and ecological investigation levels (EIL). HILs and EILs are meant as screening criteria for soil contaminants above which further investigation is needed for more site-specific qualitative or quantitative risk assessment, for example, in accordance with the national health risk assessment framework (enHealth 2004). The data obtained from this risk-based approach are related to proposed land uses and conditions that may be needed for contaminant remediation or ongoing management of site contamination. In the absence of site-specific data, the bioavailability of a contaminant is assumed to be 100 %. This conservative assumption could lead to unnecessary or expensive remediation options. This review specifically addresses the importance of considering bioavailability as a

key parameter which can be utilized for minimising the uncertainty associated with exposure in risk assessment.

The aim was to review current bioavailability and bioaccessibility approaches, methods and limitation to provide a general guidance in the NEPM for determining their use and application in contaminated site assessment with a focus on human health risk assessment, with particular reference to lead (Pb) and arsenic (As).

The scope of this review was to deliver the following outcomes:

1. Provide acceptable and relevant definitions related to bioavailability and associated parameters such as relative bioavailability and oral bioaccessibility
2. Review and contrast national and international approaches to in vitro and in vivo testing to assess human health exposure
3. Identify acceptable bioavailability testing methods for soil contaminants in Australian jurisdictions including related bioaccessibility testing and quality assurance and quality control (QA/QC) of procedures
4. Discuss the application and limitations of methods including the effects of soil pH and clay content in bioavailability–bioaccessibility assessment
5. Provide practical information on when and how bioavailability testing should be considered using a risk-based tiered framework that includes economic and land use considerations. The framework must ensure that bioavailability–bioaccessibility considerations are only undertaken when required in the NEPM risk-based approach to ensure that testing is not unnecessarily mandated in the regulation of site assessment.

Definition of bioavailability and bioaccessibility

The terms for bioavailability and bioaccessibility have often been intermixed in the literature (Naidu et al. 2008a, b). Therefore, it is necessary to have a clear understanding of the bioavailability and bioaccessibility processes and their respective definitions. In light of the differing viewpoints on the definition of bioavailability, the Committee on Bioavailability of Contaminants in Soils and Sediments (NRC 2003) prefers to use the term 'bioavailability processes' to encapsulate the mechanisms involved in the dissolution, transport and absorption of environmental contaminants by a receptor organism. Figure 1 provides a visual representation of the term bioavailability that encompasses processes from A to D. Bioavailability processes are defined (NRC 2003) as the individual physical, chemical and biological interactions that determine the exposure of plants and animals to chemicals associated with soils and sediments. In the broadest sense, bioavailability processes describe a chemical's ability to interact with the biological world, and they are quantifiable through the use of multiple tools. Bioavailability processes incorporate a number of steps, not all of which are significant for all contaminants or all settings, and there are barriers that change exposure at each step. Thus, bioavailability processes modify the amount of chemical in a soil or sediment that is actually absorbed and available to cause a biological response. Details of each compartmental process are described in the NRC document (NRC 2003). Presently, our understanding of the mechanistic bioavailability processes in Fig. 1 is highly variable, and quantitative descriptive models of bioavailability processes in most cases are lacking (perhaps with the exception of Pb bioavailability).

In the clinical and pharmacology world, the definition of bioavailability is clear and is used to describe the fraction of an administered dose of an unchanged drug (parent compound) that reaches systemic circulation. When this compound is administered intravenously, its bioavailability is arbitrarily 100 %. However, when the same compound is administered via other routes (such as orally), its bioavailability decreases (due to incomplete absorption and first-pass metabolism). Bioavailability is

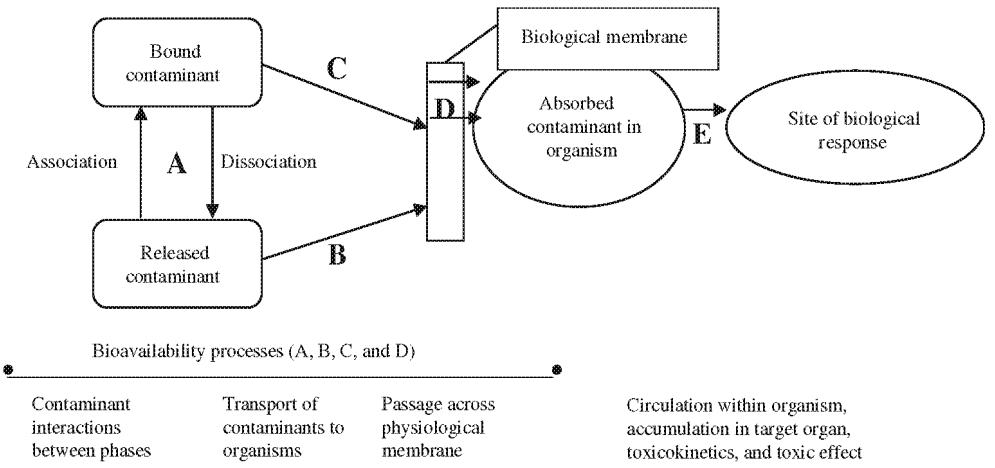

Fig. 1 Bioavailability processes in soil or sediment, including the release of a solid-bound contaminant and subsequent transport, direct contact of a bound contaminant, uptake by passage through a membrane and incorporation into a living system. Note that *A*, *B* and *C* can occur internal to an organism, such as in the lumen of the gut (redrawn from NRC 2003)

one of the essential tools in pharmacokinetics as bioavailability must be considered when calculating the dosages for non-intravenous routes of administration. This definition under the clinical setting has since been widely adopted by other science disciplines, including environmental science. Many variations of bioavailability definition have been described (NRC 2003). Taking into consideration multiple exposure pathways and in the context of environmental contamination assessment, bioavailability is broadly defined as follows:

"*Bioavailability* is the amount of a contaminant that is absorbed into the body following skin contact, ingestion, or inhalation". Within this general definition, more specific bioavailability definitions are derived, as follows:

- *Absolute bioavailability* is the fraction or percentage of a compound which is ingested, inhaled or applied to the skin that is actually absorbed and reaches systemic circulation.
- *Relative bioavailability* is the ratio of the absorbed fraction from the exposure medium in the risk assessment (e.g. soil) to the absorbed fraction from the dosing medium used in the critical toxicity study.

Whereas contaminant bioaccessibility is defined as follows:

"*Bioaccessibility* is the fraction of a contaminant that is soluble in the gastrointestinal tract and is therefore available for absorption", which is specifically referred to when in vitro assessment models are used (Kramer and Ryan 2000; Rodriguez et al. 1999; Ruby et al. 1996, 1999).

National and international approaches

Similar to the approach adopted by many environmental regulatory agencies worldwide, the Australian NEPM HILs are generally set using a conservative default of 100 % contaminant bioavailability (NEPC 1999). Although there is general acceptance that contaminant bioavailability is an important consideration in the site assessment process, there is considerable reticence to include bioavailability–bioaccessibility assessment into regulatory guidelines (Naidu and Bolan 2008; Naidu et al. 2008a, 2013a, b). Typically, this lack of acceptance is based on the following reasons:

- Bioaccessibility–bioaccessibility results depend on the assessment method used, the soil type and the contaminant.
- A method developed and validated for one contaminant is not always appropriate for other contaminants.
- A method developed and validated for one soil type is not always appropriate for other soil types.
- There are no standard reference materials that can be used to validate the results from bioavailability–bioaccessibility tests or to check their reproducibility.

Furthermore, most countries do not advocate the incorporation of in vitro bioaccessibility data into risk assessment without supporting evidence from in vivo testing.

Specific analysis of international regulatory guidelines show that

- Canadian regulatory guidance allows the incorporation of bioavailability data from in vivo methods as a more accurate risk assessment on a site-specific basis. Canadian regulators have yet to issue guidance on the use of in vitro bioaccessibility methods for assessing contaminant bioaccessibility.
- Dutch researchers at the National Institute for Public Health and the Environment (RIVM) have recommended the RIVM in vitro method for use in site-specific risk assessments for lead. However, no decision on the inclusion of in vitro methodologies has been made in the Netherlands by regulatory authorities.
- The Environment Agency responsible for contamination risk assessment guidelines in the UK has extensively reviewed the current literature and decided not to incorporate bioaccessibility guidelines into risk assessment policy until scientific evidence shows that in vitro data correlate with in vivo data for contaminants and bioaccessibility methodologies are shown to be robust and reproducible.
- In Denmark, Danish regulators support the use of in vitro methods for lead bioaccessibility, but only to complement the current risk assessment tools. No formal policy, position or guidance is publicly available.
- The USEPA is the only regulatory agency that endorses an in vitro methodology that correlates with in vivo studies for lead-contaminated soils. The methodology is based on the correlation between in vivo and in vitro studies for 19 lead-contaminated soils, and a standard operating procedure has been defined for the in vitro assay (USEPA 2008, 2009).
- Lack of regulatory acceptance of in vitro methods has not limited research into assessing soil contaminant bioaccessibility issues as this is an active area of scientific research. There are several large international collaborations aimed at improving the understanding of the scientific validity of in vitro research. These include Bioaccessibility Research Canada, the Bioaccessibility Research Group of Europe (BARGE) and the Solubility/Bioaccessibility Research Consortium (SBRC, USA). For example, BARGE with the UBM test (unified BARGE method; Denys et al. 2012), PBET (Ruby et

al. 1996), SBRC (Kelley et al. 2002) and in vitro gastrointestinal (IVG; Rodriguez et al. 1999; Ng et al. 2010) are some of the more widely accepted in vitro methods for assessing the bioaccessibility of inorganic elements including Cd, Pb and As. In Australia, in vitro and in vivo measurements have gradually gained acceptance; the decision tree (see Fig. 2) and the essence of considering bioavailability and validated bioaccessibility approaches as part of health risk assessment are potentially to be adopted nationally.

Exposure pathways

Exposure pathways to metals and metalloids include inhalation, oral ingestion or dermal absorption. Of these three pathways, soil ingestion is often the major exposure pathway when the contaminant levels are high relative to that of food and other sources. Exposure to contaminants may affect various metabolic processes including those at the cellular level. Specific examples are given in the context of understanding bioavailability and bioaccessibility as the following. It aims to highlight methodologies available for the in vivo assessment of contaminant bioavailability and the development and implementation of surrogate assays (in vitro) for its determination.

Ingestion and release of contaminants from the soil matrix

Following incidental ingestion of contaminated soil, a number of digestive processes may lead to the release of contaminants from the soil matrix and potential absorption into systemic circulation. Initially ingested material is exposed to a variety of saliva enzymes (e.g. amylase, lipase) in the oral cavity.

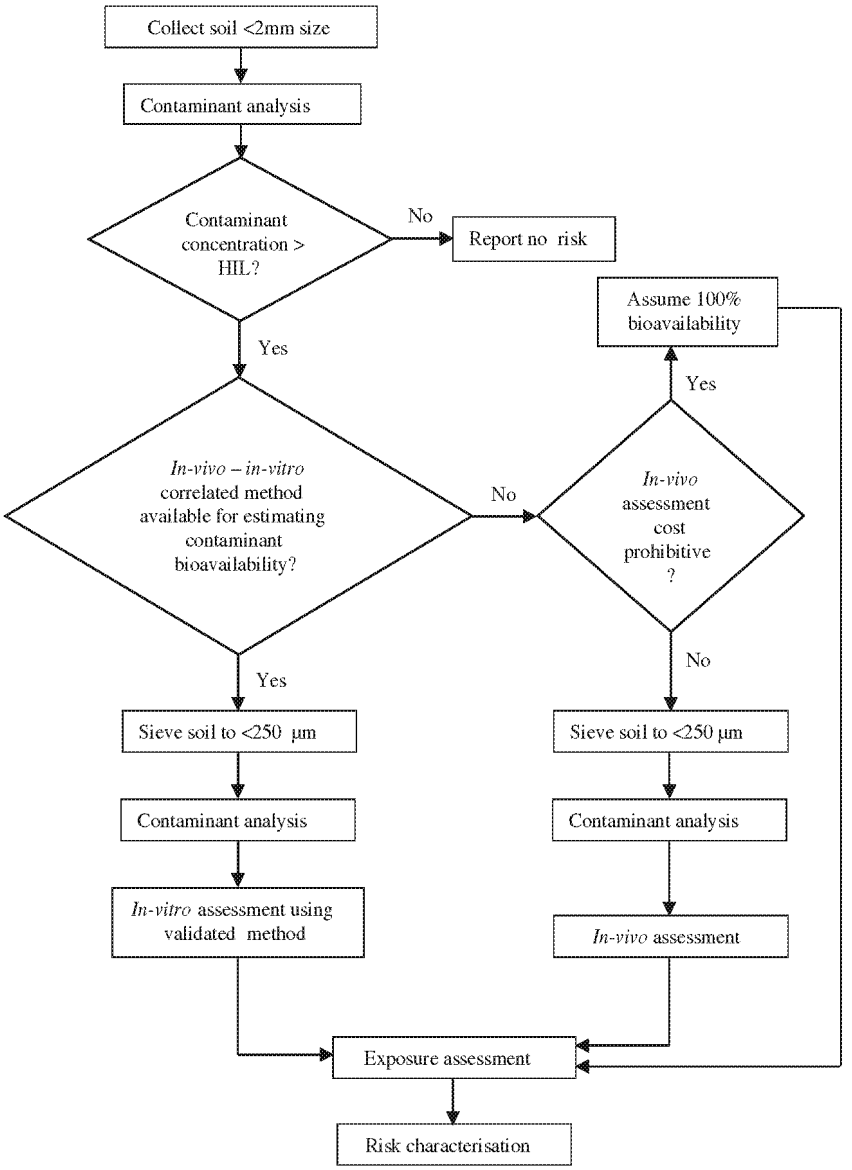

Fig. 2 Schematic diagram for the determination of contaminant bioavailability and bioaccessibility for human health risk assessment

Following mastication, the ingested material passes into the stomach via the oesophagus canal as a result of peristaltic action. In the stomach, the material is exposed to a low-pH environment (pH 1–4, depending on the degree of fasting) as a result of the secretion of hydrochloric acid from the stomach cell wall mucous membrane under gastric condition (Johnson 2001). Following transition of the chyme (partially digested food, hydrochloric acid and enzymes) from the stomach to the duodenum, bile and pancreatin are secreted from the gall bladder and pancreas, respectively, whilst bicarbonate in pancreatin juices neutralises the pH of the small intestine (Johnson 2001). In addition, other enzymes such as pepsin, amylases, lipases, proteases and nucleases are secreted by the pancreas for the breakdown of carbohydrates, fats and proteins (Gorelick and Jamieson 1994).

From the duodenum, the chyme passes through the ileum and jejunum, respectively. During this transition, the chyme is in contact with enterocytes, the most common epithelial cells that are principally responsible for fat, carbohydrate, protein, calcium, iron, vitamin, water and electrolyte absorption (Hillgren et al. 1995). Each enterocyte contains several microvilli (Madara and Trier 1994), which results in a large surface area (approximately 200 m^2 for adults) for absorption within the small intestines (Tso 1994).

Cellular interaction

In cells, there are mechanisms for metal ion homeostasis that involves a balance between uptake and efflux in order to maintain normal bodily physiological functions. Metal transporters have been periodically discovered that transport metals across cell membranes and organelles inside the cells. The importance of metal transporters is best illustrated by copper transport proteins involved in Menkes disease (copper deficiency) and Wilson disease (copper overload).

A uniform mechanism for all toxic metals is implausible because of their wide variety of chemical and toxic properties. In general terms, metals in their ionic form can be very reactive and interact with biological systems in various ways. For example, cadmium and mercury readily bind to sulphur in proteins (cysteine sulphur of amino acid residues) as a preferred bioligand, resulting in the dysfunction of biomolecules (Kasprzak 2002). The preferential binding of thiol groups can also inhibit the normal function of many enzymes in the body. One of the best-known examples is the way Pb interacts with enzymes involved in the haem synthesis pathways and results in the alteration of porphyrin profile (Moore et al. 1987). Similarly, arsenic can also interfere with haem pathways (Krishnamohan et al. 2007a, b; Ng et al. 2005).

Metals can also exert their toxicity via mimicry of essential elements by binding to physiological sites that are normally reserved for essential elements. For example,

- Cadmium, copper and nickel can mimic zinc.
- Thallium can mimic potassium.
- Manganese can mimic iron.
- Arsenate and vanadates can mimic phosphate.
- Selenate, molybdate and chromate can mimic sulphate and compete for sulphate transporters and in chemical sulphation reaction (Bridges and Zalpus 2005).

Ionic metals can form adducts with DNA and protein molecules. For example, once Cr^{VI} is absorbed (entered the cells), it can be reduced to reactive Cr^{III} species that form adducts with DNA or protein. If the adducts are not repaired, it could result in mutagenicity (Zhitkovich 2005).

Metals can directly act on catalytic centres for redox reactions and induce oxidative modification of biomolecules such as proteins or DNA. This may be a significant pathway leading to carcinogenicity by certain metals (Kasprzak 2002). On the other hand, some metals/metalloids such as arsenic can produce free radicals during their metabolism and result in oxidative damage (Wang et al. 2009), mutagenesis (Ng et al. 2001) and carcinogenesis (Krishnamohan 2008) of the receptor. The metabolism of arsenic can also explain its inhibitory effect on DNA repairs (Tran et al. 2002). More recently, it has been shown that arsenic exerts its oxidative effect by interfering with the bilirubin antioxidation defence mechanism (Arthur et al. 2012), which is in turn regulated by the human cytochrome P450 2A6 (Abu Bakar et al. 2012).

Methods for the determination of bioavailability

In order to determine the bioavailability of contaminants in soil for human health exposure assessment, in vivo assays utilising animal models have been applied. For inorganic contaminants, mice, rats, rabbits, dogs, swine, cattle and primates have been used. Being closely related to man, primates are the first choice for bioavailability studies; however, the cost associated with their use is prohibitive (Rees et al. 2009). Young swine are considered to be a good physiological model for the gastrointestinal absorption of contaminants in children (Weis and La Velle 1991). An outline of the advantages of utilising swine for contaminant bioavailability assessment is provided in Weis and La Velle (1991). Rodents are the most commonly used vertebrate species for bioavailability studies because of their availability, size, low cost and ease of handling. However, the endpoint used for the assessment of contaminant bioavailability will be influenced by the animal model. For example, only single blood samples may be appropriate/feasible for experiments conducted with small laboratory rodents (e.g. mice), whilst repeated blood sampling may be suitable for swine and primate studies.

Bioavailability endpoints may include the determination of the contaminant in blood, organs, urine and faeces, urinary metabolites (e.g. methylated arsenicals), DNA adducts and enzyme induction (e.g. cytochrome P450 monooxygenases).

In brief, the common endpoints used to monitor bioavailability include

- Blood (e.g. arsenic, lead)
- Urinary excretion (e.g. arsenic)
- Faecal excretion (mass balance studies, applicable to all metals and metalloids)
- Target organs (liver and kidney for lead and cadmium, bone for lead)

No single method of in vivo bioavailability has been identified as being suitable for all inorganic contaminants, and the methodologies selected are dependent on the contaminant of interest and the resource available to undertake the bioavailability studies. A considerable amount of research has been undertaken on inorganic contaminant bioavailability. The two most commonly studied inorganic contaminants are As and Pb.

Arsenic bioavailability

The absorption coefficient for As has been reported as 0.98 with a range of 0.70–0.98 (Owen 1990), suggesting that soluble arsenical compounds are readily accessible for uptake by a receptor. Both pentavalent and trivalent As are rapidly and extensively absorbed from the gastrointestinal tract of common laboratory animals following a single oral dose (see Table 1).

Based on the mouse data of Vahter and Norin (1980), arsenite is more extensively absorbed from the gastrointestinal (GI) tract compared to arsenate at lower doses (e.g. 0.4 mg As kg^{-1}), whereas the reverse is true at higher doses (e.g. 4.0 mg As kg^{-1}). The latter is consistent with the greater whole body retention of arsenite compared to arsenate at higher doses. As demonstrated in a small number of Australian studies (Ng and Moore 1996; Ng et al. 1998, 2003a, b), arsenic speciation of soil is important so that comparison to an appropriate positive control can be made when calculating bioavailability. Mixed speciation of As^{III} and As^{V} could be found in some soils, and the combined relative bioavailability would have to be determined (Ng and Moore 1996; Ng et al. 1998, 2003b). These studies also illustrate that bioavailability is dependent on solubility and the parent compounds, including As species sodium arsenate, sodium arsenite and calcium arsenite. Ng and Moore (1996) reported in a rat study that arsenic-contaminated soils obtained from formal cattle tick dip sites contained significant amounts of arsenite, and most likely in the calcium arsenite form. Liming was a general practice to precipitate arsenical compounds in the dip bath, forming calcium

Table 1 Cumulative 48-h elimination (per cent of dose) of arsenic in the urine and faeces of laboratory animals following oral and parenteral administration of inorganic arsenic

Species	Arsenic form	Dose	Route	Urine	Faeces	Total	Reference
Rat	Arsenic acid	5 mg/kg	Oral	17.2	33.0	50.2	Odanaka et al. (1980)
		1 mg/kg	i.v.	51.0	0.8	51.8	
Hamster	Arsenic acid	5 mg/kg	Oral	43.8	44.1	87.9	Odanaka et al. (1980)
		1 mg/kg	i.v.	83.9	4.0	87.9	
Hamster	Arsenic trioxide	4.5 mg/kg	Oral	43.5	9.4	52.9	Yamauchi and Yamamura (1985)
Mouse	Arsenic acid	5 mg/kg	Oral	48.5	48.8	97.3	Odanaka et al. (1980)
		1 mg/kg	i.v.	86.9	2.6	89.5	
Mouse	Sodium arsenate	0.4 mg As kg^{-1}	s.c.	86	6.4	92.4	Vahter and Norin (1980)
		0.4 mg As kg^{-1}	Oral	77	8.0	85	
		4.0 mg As kg^{-1}	Oral	89	6.1	95.1	
Mouse	Sodium arsenite	0.4 mg As kg^{-1}	s.c.	73	3.8	76.8	Vahter and Norin (1980)
		0.4 mg As kg^{-1}	Oral	90	7.1	97.1	
		4.0 mg As kg^{-1}	Oral	65	9.1	74.1	
Mouse	Sodium arsenate	0.00012 mg As kg^{-1}	Oral	65.0	16.5	81.5	Hughes et al. (1994)
		0.0012 mg As kg^{-1}	Oral	68.3	13.5	81.8	
		0.012 mg As kg^{-1}	Oral	72.1	10.5	82.6	
		0.12 mg As kg^{-1}	Oral	71.0	14.6	85.6	
		1.2 mg As kg^{-1}	Oral	68.7	18.2	86.9	
Rabbit	Sodium arsenite	0.050 mg As kg^{-1}	i.p.	75.7	9.9	85.6	Marafante et al. (1982)

i.v. intravenous, *s.c.* subcutaneous

arsenite for disposal purpose. This relatively insoluble trivalent arsenical remains unchanged in the soil for several decades, in contrast to the generalisation that arsenite converts to arsenate readily in the soil. The study demonstrates that the bioavailability of sodium arsenite, sodium arsenate and calcium arsenite differs. Soil containing natural mineral phase could also contain a significant proportion of arsenite (Ng et al. 1998). It is important, in this case, to include positive control groups of rats dosed with these different arsenical compounds separately.

Studies using mice conducted by Odanaka et al. (1980) suggest that much less As^V is absorbed from the GI tract following oral administration compared to the results of Vahter and Norin (1980): 48.5 % (5 mg kg^{-1}) compared to 89 % of the dose (4 mg kg^{-1}) in urine. This difference may be attributable to the fact that the mice in the study of Vahter and Norin (1980) were not fed for at least 2 h before and 48 h after dosing, whereas the mice in the studies of Odanaka et al. (1980) were not food-restricted. Kenyon et al. (1997) found that feeding a diet lower in fibre or 'bulk' to female B6C3F1 mice increased the absorption of As^V by ~10 % compared to standard rodent chow diet. The bioavailability of As from soils has been assessed using various animal models because this can be a significant issue in risk assessment for contaminated industrial sites where there is potential for arsenic exposure via soil ingestion. Absolute bioavailability and relative (comparative) bioavailability data are summarised in Tables 2 and 3. These studies indicate that oral bioavailability of arsenic in a soil or dust is considerably lower compared to the pure soluble salts typically used in toxicity studies. Davis et al. (1992) have pointed out that this is due mainly to the mineral content which controls solubility in the gastrointestinal tract, such as the solubility of the As-bearing mineral itself and encapsulation within insoluble matrices (e.g. silica). It is worthy to note that a fasting human stomach has a pH of about 1.3–1.5, a higher pH of 2.5 for a partially fed and 4.5 for a fully fed stomach, respectively. The small intestinal tract has a near-neutral pH of about 7. Obviously, these pH values alone will influence the solubility of contaminants throughout the digestive system once ingested.

In a recent study conducted with Australian soils, Juhasz et al. (2007b) utilised an in vivo swine assay for the determination of As bioavailability in contaminated soils. Arsenic bioavailability was assessed using pharmacokinetic analysis encompassing area under the blood plasma–arsenic concentration time curve following zero correction and dose normalisation. In contaminated soil studies, As uptake into systemic circulation was compared to an arsenate oral dose and expressed as As relative bioavailability. Arsenic relative bioavailability ranged from 6.9±5.0 to 74.7±11.2 % in 12 contaminated soils collected from former railway corridors, dip sites, mine sites and naturally elevated gossan soils. Arsenic relative bioavailability was generally low in the gossan soils and highest in the railway soils, ranging from 12.1±8.5 to 16.4±9.1 % and from 11.2±4.7 to 74.7±11.2 %, respectively.

In a follow-up study, Juhasz et al. (2008) assessed As relative bioavailability in spiked soils aged up to 12 months using in vitro and in vivo methodologies. Ageing (natural attenuation) of spiked soils resulted in a decline in in vivo arsenic relative bioavailability (swine assay) of over 75 % in soil A (*Red Ferrosol*), but had no significant effect on in vivo arsenic relative bioavailability even after 12 months of ageing in soil B (*Brown Chromosol*). Sequential fractionation, however, indicated that there was a repartitioning of As within the soil fractions extracted during the time course investigated. In soil A, the As fraction associated with the more weakly bound soil fractions decreased, whilst the residual fraction increased from 12 to 35 %. In contrast, little repartitioning of arsenic was observed in soil B, indicating that natural attenuation may only be applicable for arsenic in soils containing specific mineralogical properties.

In results similar to those found using experimental animals, As ingestion studies in humans indicate that both As^{III} and As^V are well absorbed from the GI tract (see Table 4). For example, Pomroy et al. (1980) reported that healthy male human volunteers excreted 62.3±4.0 % of a 0.06-ng dose of ^{74}As-arsenic acid (As^V) in urine over a period of 7 days, whereas only 6.1±2.8 % of the dose was excreted in the faeces. Few other controlled human ingestion studies have actually reported data on both urine and faecal elimination of As. However, between 45 and 75 % of the dose of various As^{III} are excreted in the urine within a few days (see Table 1), which suggests that gastrointestinal absorption is both relatively rapid and extensive.

Lead bioavailability

The absorption coefficient of Pb is from 0.01 to 0.14 (mean, 0.10; Owen 1990). A key study on the absorption and retention of Pb by infants was reported in 1978 (Ziegler et al. 1978). The authors conducted 89 metabolic balance studies with 12 normal infants aged 14–746 days over 72 h. Subjects were fed milk or formula and commercially prepared strained foods. The estimated Pb intakes were 0.83–22.61 (9.44±5.27) μg kg^{-1} day^{-1}. Faecal and urinary excretions of Pb were measured and net absorption and retention were calculated. Out of all the balance studies—61 studies with intakes of >5 μg kg^{-1} day^{-1}—the averaged net absorption was 41.5 % of Pb intake and net retention was 31.7 % of intake. Both absorption and retention were inversely correlated with intake of calcium, suggesting that dietary calcium reduces lead absorption. Seven of 28 studies with Pb <5 μg kg^{-1} day^{-1} and in only 3 of 61 studies at higher intakes returned a higher faecal excretion than the

Table 2 Absolute (comparison to intravenous route) oral bioavailability of arsenic from soil in laboratory animals

Species	Duration (h)	i.v. dose	Soil or sample	Soil dose	Bioavailability (%, mean ± SD)	Method	Reference
International data							
Beagle dog	120	2 mg As^{5+}	Netherlands bog ore	6.6–7.0 mg As	8.3±2.0	AUC for urinary excretion	Groen et al. (1994)
New Zealand white rabbit	120	1.95 mg As^{5+} kg^{-1}	Smelter-impacted soil (Anaconda, Montana USA)	0.78 mg As kg^{-1}	24±3.2	AUC for urinary excretion, no dose dependency observed	Freeman et al. (1993a)
			Sodium arsenate	1.95 mg As kg^{-1}			
				3.9 mg As kg^{-1}			
				1.95 mg/kg	50±5.7		
Cynomolgus monkey	168	0.62 mg As^{5+} kg^{-1}	Soil (Anaconda, Montana USA)	0.62 mg As kg^{-1}	14 (11)	AUC for urinary excretion (AUC for blood in parentheses)	Freeman et al. (1995)
			House dust (same location)	0.26 mg As kg^{-1}	19 (10)		
			Sodium arsenate	0.62 mg As kg^{-1}	68 (91)		
Immature swine	144	0.01–0.31 mg As kg^{-1}	Soil	0.04–0.24 mg As kg^{-1}	52	AUC for blood	USEPA (1996)
			Slag	0.61–1.52 mg As kg^{-1}	28		
			Sodium arsenate	0.01–0.11 mg As kg^{-1}	68		
Australian data							
Wistar rat	96	0.5 mg As^{3+} kg^{-1}	Soil (Canberra, Australia)	0.5 mg As kg^{-1} (n=6)	4.31–9.87[a] 1.27–2.98[b]	AUC for urinary excretion	Ng et al. (1998)
		0.5 mg As^{5+} kg^{-1}		5 mg As kg^{-1} (n=4)	1.02–1.96[a] 0.26–0.67[b]		
Sprague–Dawley rat	168	0.5 mg As^{3+} kg^{-1} 0.5 mg As^{5+} kg^{-1}	Gold mine tailings, waste rock, heap leach material; former arsenic mine mixed waste	0.5 mg As kg^{-1} (n=4)	0.5±0.14–8.0±1.2	AUC for urinary excretion	Bruce (2004)
Cattle	240	0.5 mg As^{3+} kg^{-1} 0.5 mg As^{5+} kg^{-1}	Gold mine tailings, waste rock, heap leach material; former arsenic mine mixed waste	1.0 mg As kg^{-1} (n=3)	7±0.34–58±6.49	AUC for blood	Bruce (2004)

n = number of composite soil samples

[a] Compared to arsenite AUC

[b] Compared to arsenate AUC

intake. Intakes <5 μg kg^{-1} day^{-1} were more likely to be unreliable. The authors speculated that infants in this treatment group were not under special study conditions and might have received intakes higher than this value. Furthermore, this study did not account for non-dietary intakes and excretion other than those with urine and faeces. Excretions via other routes (e.g. sweat) are considered to be low. However, intakes from other sources could be significant, including inhalation of fine dust and ingestion of dust with hand-to-mouth activity. If the actual intakes were higher than those measured by the authors, then the real absorption could be lower than the reported value here.

In an earlier report from 11 balance studies carried out with eight subjects aged 3 months to 8 years with intakes ranging about 5–17 μg kg^{-1} day^{-1} (mean, 10.6 μg kg^{-1} day^{-1}), the average absorption was 53 % of the intake and average retention was 18 % of the intake (Alexander et al. 1974). Lead radioisotope studies reported about 10 % absorption of Pb by human adults (Hursh and Suomela 1968; Rabinowitz et al. 1976).

Measurements of Pb relative bioavailability are often low, with ranges from as low as 6 % for New Zealand White rabbits fed contaminated soils (Davis et al. 1992) and 10.7 % observed in monkeys, also fed contaminated soils (Roberts et al. 2002). The US Environmental Protection Agency (USEPA) views only in vivo studies

Table 3 Relative bioavailability (comparison to oral gavage) of arsenic from soil in laboratory animals by comparison of blood concentrations otherwise specified

Species	Duration (h)	Oral gavage	Soil or sample	Soil dose	Bioavailability (%, mean ± SD)	Reference
International data						
New Zealand white rabbit	120	1.95 mg As^{5+} kg^{-1}	Smelter-impacted soil (Anaconda, Montana USA)	0.78 mg As kg^{-1} 1.95 mg As kg^{-1} 3.9 mg As kg^{-1}	50±5.7	Freeman et al. (1993a)
New Zealand white rabbit	120	1.35 mg As^{5+} kg^{-1}	Smelter-impacted soil (Anaconda, Montana USA)	1.25 mg As kg^{-1}	70	Freeman et al. (1993b)
Cynomolgus monkey	120	1.35 mg As^{5+} kg^{-1}	Smelter-impacted soil (Anaconda, Montana USA)	1.25 mg As kg^{-1}	43.6	Freeman et al. (1993b)
	168	0.62 mg As^{5+} kg^{-1}	Smelter-impacted soil (Anaconda, Montana USA)	0.62 mg As kg^{-1}	10–30	Freeman et al. (1995)
			Dust (same location)	0.26	10–30	
Immature swine	144	0.01–0.31 mg As kg^{-1}	Soil	0.04–0.24 mg As kg^{-1}	78	USEPA (1996)
			Slag	0.61–1.52 mg As kg^{-1}	42	
Australian data						
Wistar rat	96	Sodium arsenite: 5 mg As^{3+} kg^{-1} Calcium arsenite: 5 mg As^{3+} kg^{-1} Sodium arsenate: 5 mg As^{5+} kg^{-1}	Soils (from 16 cattle dip sites, NSW, Australia)	5 mg As kg^{-1}	8.1±4.0 14.4±7.1 60±32.4	Ng and Moore (1996)
	96	Sodium arsenite: 0.5 mg As^{3+} kg^{-1} Calcium arsenite: 0.5 mg As^{3+} kg^{-1} Sodium arsenate: 0.5 mg As^{5+} kg^{-1}	Soils (9 samples from one CCA-contaminated site)	0.5 mg As kg^{-1}	13.0±4.5 32.2±11.2 38±13.2	Ng and Moore (1996)
Sprague–Dawley rat	168	Sodium arsenite: 5 mg As^{3+} kg^{-1} Sodium arsenate: 5 mg As^{5+} kg^{-1}	Gold mine tailings, waste rock, heap leach material; former arsenic mine mixed waste	0.5 mg As kg^{-1}	1±0.28–20±3.09 (urine)	Bruce (2004)
	240	Sodium arsenite: 5 mg As^{3+} kg^{-1} Sodium arsenate: 5 mg As^{5+} kg^{-1}	4 categories of mine wastes	0.5 mg As kg^{-1}	1.6–8.9 %	Diacomanolis et al. (2007)
Swine (20–25 kg b.w.)	26	Sodium arsenate: 0.1 mg As^{5+} kg^{-1}	Herbicide/pesticide impacted, mine site and gossan soils (n= 12)	0.03–0.4 mg As kg^{-1}	6.9±5.0–74.7±11.2 %	Juhasz et al. (2007b)
	26	Sodium arsenate: 0.1 mg As^{5+} kg^{-1}	Red Ferrosol soil[a]	0.2–0.25 mg As kg^{-1}	53±33.9–24.1±9.5 g	Juhasz et al. (2008)
			Brown Chromosol soil[b]	0.2–0.5 mg As kg^{-1}	97.2±1.9–92.9±5.3 11±0.6–69±7.24	

Table 3 (continued)

Species	Duration (h)	Oral gavage	Soil or sample	Soil dose	Bioavailability (%, mean ± SD)	Reference
Cattle	266 days (repeated doses)	Sodium arsenite: 0.5 mg As^{3+} kg^{-1} Sodium arsenate: 0.5 mg As^{5+} kg^{-1}	Gold mine tailings, waste rock, heap leach material; former arsenic mine mixed waste	0.5 mg As kg^{-1}	2±0.3–29±19 (liver)	Bruce et al. 2003; Bruce (2004)

Where in Juhasz et al. 2008: both soils (Red Ferrosol and Brown Chromosol) were aged for 12 months. The bioavailability data showed the decrease of bioavailability from 0 to 12 months after ageing. Data from 3 and 6 months are not shown here

[a] Red Ferrosol soil contained 47.5 % sand, 24 % clay and 20 % silt, spiked with sodium arsenate to achieve a final arsenic concentration of 1,000 mg/kg (Juhasz et al. 2008)

[b] Brown Chromosol soil contained 87 % sand, 7.8 % clay and 2.7 % silt, spiked with sodium arsenate to achieve a final arsenic concentration of 1,000 mg/kg (Juhasz et al. 2008)

conducted using the juvenile swine as the appropriate animal to undertake lead studies to estimate lead bioavailability in young children. The USEPA assumed that 30 % of ingested Pb in soil will be bioavailable (absolute bioavailability) when assessing lead bioavailability of contaminated sites (USEPA 1994). However, recent bioavailability studies using the animal models mentioned above have demonstrated that the BA of lead from some soils and mine waste materials may indeed be considerably lower or higher, as confirmed in later studies.

Multiple bioavailability studies done by the USEPA Region 8 on 18 soil and soil-like samples and one NIST paint material using the juvenile swine model were reported recently (USEPA 2007b). Although the gastrointestinal tract of immature swine is believed to be similar to that of young humans, the absolute bioavailability of lead acetate was found to be 10, 14, 16 and 19 % as measured using blood (area under the curve, AUC), femur, liver and kidney, respectively (average, 15±4 %). Lead absorption in juvenile swine is apparently lower than for a young human (42–53 %; see below). The relative bioavailability (RBA) varied widely between different test materials, with the lowest RBA of about 1 % in California Gulch Oregon Gulch tailings and the highest of 105 % in California Gulch Fe/Mn PbO. Galena-enriched soil (PbS) had the lowest RBA range of −1 to 4 %. The report concluded that the wide variability highlights the importance of obtaining and applying reliable RBA data in order to help improve risk assessment for lead exposure. Although available data are not yet sufficient to establish reliable quantitative estimates of RBA for each of the different mineral phases of lead in the test materials, the report provides a tentative ranking order of the phases into three semiquantitative categories (low, medium or high RBA), as shown in Table 5.

In a recent study by Juhasz et al. (2009a, b), in vivo swine experiments were performed to determine the relative bioavailability of Pb in (Australian) contaminated soils. Lead doses were administered under fasting conditions to represent a worst-case scenario for Pb exposure. Feed was administered to swine 2 h after Pb dosage to ensure the lowest gastric conditions at the time of Pb exposure. The relative bioavailability of Pb in contaminated soils was determined by comparing the area under the blood–Pb concentration time curve for orally administered contaminated soil and a Pb acetate reference dose. The mean Pb relative bioavailability for contaminated soils ranged from 10.1±8.7 to 19.1±14.9 %. Variability in Pb relative bioavailability was observed between triplicate soil analysis due to physiological intraspecies differences including genetic factors, disparity in stomach clearance times, stomach pH and the rate of Pb absorption. Previous Pb relative bioavailability assays utilising swine have reported Pb relative bioavailability in contaminated soils to range from 1 to 87 % (Marschner et al. 2006; Schroder et al. 2004),

Table 4 Metabolism and urinary excretion of inorganic and organic arsenicals in human following experimental administration

Form	Adult subjects	Dose and frequency	Time duration (days)	% dose in urine	% of total urinary metabolites				Reference
					As^{5+}	As^{3+}	MMA	DMA	
Arsenic acid	6	0.01 μg	5	57.9		27.2	20.6	51.0	Tam et al. (1979)
Arsenic acid	6	0.06 ng	7	62.3		ND			Pomroy et al. (1980)
Arsenic trioxide	1	700 μg	3	68.2	7.9	31.7	28.2	32.2	Yamauchi and Yamamura (1979)
Sodium arsenite	3	500 μg	4	45.1	25		21.3	53.7	Buchet et al. (1981a)
Sodium metaarsenite	1	125 μg×5 days	14	54	16		34	50	Yamauchi and Yamamura (1979)
	1	250 μg×5 days	14	73	7		20	73	
	1	500 μg×5 days	14	74	19		21	60	
	1	1,000 μg× 5 days	14	64	26		32	42	
Sodium mono-methyl arsonate	4	500 μg	4	78.3	ND	ND	87.4	12.6	Buchet et al. (1981a)
Sodium dimethyl arsinate	4	500 μg	4	75.1	ND	ND	ND	100	Buchet et al. (1981b)

ND not determined

although in the study of Marschner et al. (2006), Pb relative bioavailability (17–63 %) was calculated following the determination of Pb in selected organs, bone and urinary excretions, but not blood analysis. Examples of bioavailability data obtained from various animal models are summarised in Tables 6 and 7.

Based on the available literature data on lead in humans, the Integrated Exposure Uptake Biokinetic Model for Pb (IEUBK) used by USEPA estimates that the absolute bioavailability of Pb from water and diet is usually about 50 % in children (USEPA IEUBK 2007). Thus, when a reliable site-specific RBA value for soil is available, it may be used to estimate a site-specific absolute bioavailability in that soil using Eq. 1.

$$ABA_{soil} = 50\% \times RBA_{soil} \quad (1)$$

Factors influencing contaminant bioavailability

The bioavailability of ingested contaminants will vary depending on the matrix in which it is ingested (e.g. food, water, beverages, soil). Indeed, the solubility of the arsenical compound itself, the presence of other food constituents and the nutrients in the gastrointestinal tract may all influence the bioavailability of As. There are other factors which may influence bioavailability. These include pH of the gastrointestinal conditions of the receptor, pH of the contaminated material, and the chemical and physical properties of the material. Furthermore, it is generally true that smaller-particle-size material has a greater bioavailability compared to larger-sized particles of the same material. Since a particle size of <250 μm is the most likely fraction ingested via hand-to-mouth activities by children (USEPA 2009), it is important that bioavailability be determined using this particle size fraction. In brief, both environmental and physiological conditions can influence the bioavailability.

Owen (1990) reported the absorption coefficients for 39 chemicals via oral and inhalation routes of exposure that could be indicative for bioavailability. Although there are differences in the physiological features and metabolisms in animals and humans, bioavailability considering only the absorption fraction via the GI tract can be determined using

Table 5 RBA ranking order found in various soil and soil-like materials obtained from juvenile swine dosing experiments (from USEPA 2007a, b, c)

Low bioavailability RBA<25 %	Medium bioavailability RBA=25–75 %	High bioavailability RBA>75 %
Fe(M) sulphate	Lead phosphate	Cerussite (lead carbonate)
Anglesite	Lead oxide	Mn(M) oxide
Galena		
Pb(M) oxide		
Fe(M) oxide		

Table 6 Absolute (comparison to intravenous route) oral bioavailability of lead in laboratory animals

Species	Duration	i.v. dose	Soil or sample	Soil dose	Bioavailability (%, mean ± SD)	Method	Reference
International data							
Humans	72 h	0.02, 0.20, 2.0 mg/kg b.w. for 29 days	Food	0.83–22.61 μg Pb kg^{-1} day^{-1} (food)	41.5	Urinary and faecal excretion	Ziegler et al. (1978)[a]
Sprague–Dawley rat	29–30 days		Lead acetate	0.08–26.2 mg Pb kg^{-1} day^{-1} (lead acetate spiked-feed)	15±8.1 (blood), 7.4±4.1 (bone), 11±7.1 (liver)	Blood, bone or liver	Freeman et al. (1994)
				0.12–23.8 mg Pb kg^{-1} day^{-1} (mine waste)	2.7±1.5 (blood), 0.4±0.16 (bone), 0.55±0.68 (liver)		
Juvenile swine	360 h	100 μg Pb kg^{-1}	Lead acetate solution	0	10–19	AUC for blood, necropsy Pb in liver, kidney or bone	USEPA (2007b)
Juvenile swine	15 days	100 μg Pb kg^{-1}	Lead acetate solution	75, 225 or 675 μg Pb kg^{-1} day^{-1} (berm or residential soil)	28 (blood) and 43 (liver) for berm soil; 29 (blood) and 37 (liver) for residential soil	AUC for blood or necropsy Pb in liver	Casteel et al. (1997)
Australian data							
Sprague–Dawley rat	240 h	0.5 mg Pb kg^{-1}	Lead acetate solution	0.01–2.7 mg Pb kg^{-1} (various mine wastes)	0.6–1.4	AUC for urinary excretion	Diacomanolis et al. (2007)
	168 h	1 mg P/kg^{-1}	Lead acetate solution	10 mg Pb kg^{-1} (zinc concentrate)	1±2.15	AUC for urinary excretion	Bruce (2004)
Cattle	240 h	0.5 mg Pb kg^{-1}	Lead acetate solution	5 mg Pb kg^{-1} (in zinc concentrate)	3±0.51	AUC for blood	Bruce (2004)

[a] This food study is included as a cross-reference to soil exposure

various animal models provided a proper positive control group of animals is included in the same study.

Ng and Moore (1996) proposed the use of a single rat blood for relative bioavailability (they also referred to comparative bioavailability in the literature). The advantage of rats is their greater capacity to bind As in the red blood cells compared to other animal species, including humans. For the same dose, the rat blood As is 60-fold greater than that of a guinea pig. They are of the view that rats are sufficiently large enough compared to mice and more cost-effective than larger mammals such as dogs, pigs and monkeys, or perhaps the ultimate human model. It has been demonstrated that the metabolism (methylation of pathway) of As is different amongst various animal species (Vahter 1994), although this is yet unclear in pigs. For example, chimpanzees and marmoset monkeys are unable to methylate arsenic (Vahter et al. 1995; Zakharyan et al. 1996). It has been often cited that there is no perfect animal model to replicate arsenic metabolism in humans. However, for bioavailability studies, given the way in which the relative bioavailability of a given metal/metalloid is operationally defined and measured, differences in one's metabolism, even if they exist, would not be a confounding factor (Roberts et al. 2002). Hence, many animal species have been employed for bioavailability studies.

Although in vivo studies utilising animal models are an appropriate method for determining contaminant bioavailability in soil for inclusion in human health exposure assessment, the time required for in vivo studies, the expense of animal trials and ethical issues preclude their use as routine bioavailability assessment tools. As a result, rapid, inexpensive in vitro methods simulating gastrointestinal conditions in the human stomach have been developed as surrogate bioavailability assays. These assays determine contaminant concentrations that are solubilised following gastrointestinal extraction and are therefore available for absorption into systemic circulation. This fraction is referred to as the 'bioaccessible fraction' and is specifically used when in vitro assessment models are used. The following sections detail the development of in vitro methods, key assay parameters and examples where the bioaccessibility of organic and inorganic contaminants in soils has been determined.

Table 7 Relative bioavailability (comparison to oral gavage) of lead from soil in laboratory animals by comparison of blood concentrations otherwise specified

Species	Duration	Oral gavage	Soil or sample	Soil dose	Bioavailability (%, mean ± SD)	Reference
International data						
Juvenile swine	360 h	0.025–0.225 mg Pb kg^{-1} day^{-1} (lead acetate)	18 soils of various mineral phases and 1 NIST paint	0.075–0.625 mg Pb kg^{-1} day^{-1}	0–105	USEPA (2007a)
Juvenile swine	15 days	0, 75, 225 μg Pb kg^{-1} day^{-1}	Berm or residential soil	75, 225 or 675 μg Pb kg^{-1} day^{-1} (berm or residential soil)	56–58 (blood), 74–86 (liver), 68–74 (kidney), 68–72 (bone)	Casteel et al. (1997)
Sprague–Dawley rat	30 days	0.076–25.7 mg Pb kg^{-1} day^{-1}	Mine waste soil	0.119–23.2 mg Pb kg^{-1} day^{-1}	12.1–26.8 (blood), 4.8–13.3 (bone), 0.6–13.6 (liver)	Freeman et al. (1992)
Australian data						
Sprague–Dawley rat	168 h	10 mg Pb kg^{-1} (lead acetate)	Zinc concentrate	10 mg Pb kg^{-1}	38±6.8	Bruce (2004)
Juvenile swine	5 days	0.025–0.225 mg Pb kg^{-1} (lead acetate)	5 soils from a domestic incinerator site and residential land developed on quarry fill material	0.225–0.53 mg Pb kg^{-1}	7.4±4.2–19.1±1.9	Juhasz et al. (2009a, b)
Cattle	240 h	5 mg/kg (lead acetate)	Zinc concentrate	5 mg Pb/kg	80±13.2	Bruce 2004
	266 days	0.5 mg Pb kg^{-1} day^{-1} (lead acetate)	Zinc concentrate	0.5 mg–Pb kg^{-1} day^{-1}	48±3.2 (liver)	Bruce (2004)

In vitro methods for the determination of bioaccessibility

In order to determine the bioaccessibility of contaminants in soil, a variety of in vitro gastrointestinal extraction methods have been developed. These methodologies attempt to simulate processes that occur in the human body that lead to the release of contaminants from the soil matrix. As the human digestive system is an extremely complex system, these methodologies do not attempt to replicate the conditions found in various compartments, but mimic key processes. The methodologies may consist of up to three phases including esophageal, gastric and intestinal. However, due to the short residence time that a material spends in the mouth (approximately 2 min), this phase may not contribute significantly to contaminant release and therefore may be optional. The gastric and intestinal phases are present in most in vitro methodologies (examples of which are given in Table 8), although compartment parameters may vary between methods.

There are several other methodologies that are sometimes utilised to assess contaminant bioaccessibility. These include methodologies to assess the potential leaching characteristics of waste material (toxicity characteristic leaching procedure: USEPA 1992; the Australian standard leaching procedure: Council of Standards Australia 1997). These methods have been developed for estimating the potential leaching characteristics of the waste material and should not be utilised to estimate contaminant bioaccessibility. Similarly, strong acids (i.e. 0.1 M HCl) have often been utilised to estimate potential metal bioaccessibility in contaminated soils. These methodologies have not been correlated with in vivo bioavailability data and have limited applicability for in vitro estimations.

When developing in vitro assays for assessing contaminant bioaccessibility, the parameters should be representative of the physiology of children, the group most at risk from exposure to environmental contaminants. Key factors to be considered for in vitro methodologies are discussed below.

Chyme composition

The composition of the gastric and intestinal phases ranges from simple systems containing few constituents (Rotard et al. 1995) to highly complex assays that contain numerous constituents, including simulated intestinal microbial communities (e.g. SHIME; Molly et al. 1993). Pepsin is a base constituent of the in vitro gastric phase. It is a digestive protease which functions to break down proteins into peptides. It has also been suggested that pepsin may decrease the surface tension of chyme (Tang et al. 2006), thereby increasing the mobilisation potential and solubility of organic constituents (Charman et al. 1997). Alternatively, glycine is utilised as the main constituent of the gastric phase instead of pepsin. Mucins are also a commonly added constituent to the gastric phase of in vitro bioaccessibility assays. Mucins are a group of large glycosylated proteins that are secreted

Table 8 Composition and in vitro parameters commonly utilised in in vitro bioaccessibility assays (SBRC, IVG, PBET and DIN) for inorganic contaminants

Method/ phase	In vitro parameters			
	Composition (g l^{-1})	pH	Soil/ solution ratio	Extraction time (h)
SBRC				
Gastric	30.03 g glycine	1.5	1:100	1
Intestinal	1.75 g bile, 0.5 g pancreatin	7.0	1:100	4
IVG				
Gastric	10 g pepsin, 8.77 g NaCl	1.8	1:150	1
Intestinal	3.5 g bile, 0.35 g pancreatin	5.5	1:150	1
PBET				
Gastric	1.25 g pepsin, 0.5 g sodium malate, 0.5 g sodium citrate, 420 µl lactic acid, 500 µl acetic acid	1.5, 2.5 and 4.0 7.0	1:100	1
Intestinal	1.75 g bile, 0.5 g pancreatin		1:100	4
DIN				
Gastric	1 g pepsin, 3 g mucin, 2.9 g NaCl, 0.7 g KCl, 0.27 g KH_2PO_4	2.0	1:50	2
Intestinal	9.0 g bile, 9.0 g pancreatin, 0.3 g trypsin, 0.3 g urea, 0.3 g KCl, 0.5 g $CaCl_2$, 0.2 g $MgCl_2$	7.5	1:100	6
BARGE UBM				
Saliva–gastric	Saliva (pH 6.5±0.5): 0.896 g KCl, 0.888 g NaH_2PO_4, 0.2 g KSCN, 0.57 g Na_2SO_4, 0.298 g NaCl, 1.8 ml of 1 M NaOH, 0.2 g urea, 0.145 g amylase, 0.05 g mucin, 0.015 g uric acid Gastric phase (pH 0.9–1.0): 2.752 g NaCl, 0.266 g NaH_2PO_4, 0.824 g KCl, 0.4 g $CaCl_2$, 0.306 g NH_4Cl, 8.3 ml of 37 % HCl, 0.65 g glucose, 0.02 mg glucuronic acid, 0.085 g urea, 0.33 g glucosaminehydrochloride, 1 g bovine serum albumin, 3 g mucin, 1 g pepsin	1.2	0.6:22.5	1
Gastric–intestinal	Duodenal phase (pH 7.4±0.2): 7.012 g NaCl, 5.607 g $NaHCO_3$, 0.08 g KH_2PO_4, 0.564 mg KCl, 0.05 g MgCl2, 0.18 ml of 37 % HCl, 0.1 g urea, 0.2 g $CaCl_2$, 1 g bovine serum albumin, 3 g pancreatin, 0.5 g lipase Bile phase (pH 8.0±0.2): 5.259 g NaCl, 5.785 g $NaHCO_3$, 0.376 g KCl, 0.18 ml of 37 % HCl, 0.25 g urea, 0.222 $CaCl_2$, 1.8 g bovine serum albumin, 6 g bile	6.5	0.6:58:5	4

on the mucosal surface. Mucins are added to in vitro gastric phases to increase contaminant mobilisation in the digestive tract (Hack and Selenka 1996). Oomen (2000), Wittsiepe et al. (2001) and Ruby et al. (2002) included protein in gastric phase solutions in order to enhance organic contaminant solubilisation from the soil matrix. Similarly, lipids (e.g. oleic acid) may be included in gastric phase solutions to increase organic contaminant solubilisation. The detergency effect of bile-ingested lipids (e.g. triglycerides) leads to the formation of mixed bile–lipid micelles. Soil-borne organics may be mobilised into these micelles, which are potentially available for absorption (Hack and Selenka 1996; Holman 2000; Oomen 2000).

Bile and pancreatin are central components of the intestine phase of in vitro bioaccessibility assays. The influence of bile on the mobilization of organic contaminants has been well established. Bile is secreted from the gall bladder into the duodenum during digestion in order to facilitate the breakdown of lipids. Consisting of cholesterol, phospholipids, bile pigments, bile salts and bicarbonate (Dean and Ma 2007), bile forms aggregates with monoglycerides and fatty acids (bile fat micelles) as a result of lipase. As a result of micelle formation, contaminant solubility may be enhanced due to the incorporation of these compounds into high surface area amphipathic molecules (Gorelick and Jamieson 1994; Walsh 1994). Whilst human bile may not be used in in vitro assays due to ethical issues (Alvaro et al. 1986; Wildgrube et al. 1986), a variety of animal bile has been used. Due to the similarity of bile salt percentage to human bile, bovine and porcine bile are considered most suitable for in vitro applications (Oomen et al. 2004). Different forms of bile have been used in various models, such as purified uniform bile salts and animal origin bile (Friedman and Nylund 1980). Previous studies identified that mixed micelles, an important parameter for absorption, may not be formed when using purified uniform bile salts. As a result, some in vitro assays suggest the use of original freeze-dried bile obtained from animals (Hack and Selenka 1996; Minekus et al. 1995; Oomen et al. 2002; Rotard et al. 1995; Ruby et al. 1996). Whilst chicken bile was used in some early in vitro assays (Rotard et al. 1995), its use has been discouraged due to its varying solubilising effects compared to other bile products. For example, Pb bioaccessibility was 3–5.5 times greater when chicken bile

was used in the intestinal phase compared to bovine and porcine bile (Oomen et al. 2003, 2004). Bovine or porcine bile is preferred to chicken bile because chicken bile may lead to an irregular and unaccountable bioaccessibility pattern, and the composition of chicken bile is significantly different from the composition of human bile.

Pancreatin is also included in simulated intestinal fluid. Pancreatin is a mixture of digestive enzymes (amylase, lipase, trypsin and protease) which hydrolyse proteins to oligopeptides (trypsin), hydrolyse starch to oligosaccharides (amylase) and hydrolyse triglycerides to fatty acids and glycerol (lipase). In some cases, trypsin is added to intestinal fluid to increase the capacity for protein hydrolysis (Hack and Selenka 1996; Wittsiepe et al. 2001).

Gastric and intestinal phase pH

The pH of the human gastric system can vary significantly depending on whether the subject is under fasting or fed states. Ruby et al. (1996) reported that the mean fasting pH value for young children ranged from 1.7 to 1.8, with a range of 1–4. Following ingestion of food, the stomach pH will rise to >4 and will return to basal levels 2 h following stomach emptying. In vitro studies (e.g. UBM) have shown that pH is a critical factor that can influence the bioaccessibility results (Pelfrêne et al. 2011a, b; Wragg et al. 2011). Developers of in vitro assays have generally chosen a gastric phase pH value which represents a worst-case scenario (fasted state) for young children. Low pH stomach values are particularly prudent for the assessment of metal bioaccessibility as the pH will drive the dissolution of metals and mineral phases, thereby controlling the fraction that is potentially available for uptake. The majority of bioaccessibility assays employ a gastric pH of 1–2. However, Molly et al. (1993) utilised a gastric phase pH of 5.2 in the SHIME method which was meant to represent an infant's fed state stomach pH. The pH in the small intestine varies from 4–4.5 in the initial part of the small intestine to 7.5 in the ileum (Daugherty and Mrsny 1999; Guyton 1991; Johnson 2001; Sips et al. 2001). Most in vitro assays adjust the pH of the intestinal phase to near neutral (6.5–7.5); however, Oomen (2000) employed a pH value of 5.5.

Gastric and intestinal phase residence time

Nutrition studies have demonstrated that complete emptying of the stomach may occur after 1–2 h, whilst 3–5 h is required for chyme to pass from the start of the small intestine to the start of the large intestine. Most bioaccessibility assays reflect the time frames with gastric extraction times ranging from 1 to 3 h and intestinal extraction times of 2–6 h. It has been proposed that small variations in residence time do not have a significant impact on bioaccessibility (Daugherty and Mrsny 1999; Degan and Philips 1996; Guyton 1991; Johnson 2001).

Soil/solution ratio

Soil/solution ratio is one parameter that exhibits the greatest variability between in vitro assays. Ratios of 1:2 g ml^{-1} up to 1:5,000 g ml^{-1} have been used by a variety of researchers. Ruby et al. (1992, 1996) proposed that assays which utilise soil/solution ratios of 1:5 to 1:25 may underestimate the bioaccessible fraction due to solubility issues at these ratios as a result of diffusion-limited dissolution kinetics. However, Hamel et al. (1998) demonstrated that little differences in metal bioaccessibility values resulted from soil/solution ratios of 1:100 to 1:5,000 g ml^{-1}. Whilst this may be the case for metal contaminants, the influence of soil/solution ratio on organic contaminant bioaccessibility has received little attention. As a result, a soil/solution ratio of 1:100 has been selected for most in vitro assays for the assessment of metal bioaccessibility as this ratio would not influence the test results. In addition, due to insufficient data for fasting children to support any soil/solution ratio, 1:100 was selected arbitrarily to represent a fasting child (Ruby et al. 1996).

Amendments to gastric and intestinal phases

Another variable between in vitro assays is the inclusion of food additives. Food may be added to in vitro assays for a comparison of contaminant bioaccessibility between the fed and fasted states. As discussed above, a stomach with food would have a higher pH that may be less favourable for metal solubility (Ruby et al. 1996). In addition, the choice and amount of food added to in vitro assays may have a significant effect on bioaccessibility measurements depending on the fat and protein contents of the additive. The inclusion of food additives has been shown to increase the bioaccessibility of organic contaminants. Hack and Selenka (1996) included lyophilised milk as part of the in vitro assay with increased PAH and PCB mobilisation from 5–40 to 40–85 %.

Other parameters

Irrespective of the methodology, bioaccessibility assays are conducted at 37 °C as this is the physiological temperature of the human body. In addition, agitation is required during bioaccessibility assessment, either in the form of shaking, stirring, end-over-end rotation, inert gas movement or peristaltic movement, in order to mimic gastrointestinal turbulence. Most in vitro methods are performed under batch condition, i.e. the simulated digestive contents are reacted and analysed in one vessel and compartments are added to the system step by step during the process. Alternatively, in order to better represent the human digestive system and its motion, a 'flow-through' model was developed by Molly et al. (1993) and Minekus et al. (1995).

Bioaccessibility of contaminated soil

Tables 9 and 10 outline the variability in As and Pb bioaccessibility when contaminated soils were assessed using a variety of in vitro methodologies. A number of parameters can influence the bioavailability of the inorganic contaminants in soils. Studies by Yang et al. (2002) identified Fe oxide content and pH to be the principal soil factors controlling arsenic bioaccessibility. Similarly, Juhasz et al. (2007a, b) identified that arsenic bioaccessibility was related to the free or total Fe content of the soil. These results were also observed by Roussel et al. (2010) and Pelfrêne et al. (2011a). Recent evidence has suggested that contaminant ageing decreases bioavailability due to changes in surface phase complexes with increasing arsenic soil residence time (Fendorf et al. 2004). Once sorbed by the soil, increasing the residence time (ageing) may lead to the development of inner sphere complexes, surface diffusion within micropores or surface precipitates (Aharoni and Sparks 1991), resulting in a decrease in As bioavailability.

Contaminant bioaccessibility may also be influenced by the in vitro methodology employed. As outlined in previous sections, the composition of the gastrointestinal fluid (i.e. bile concentration, inclusion of food additives, etc.) can significantly influence the release of contaminants from the soil matrix. Whilst round-robin studies have been undertaken to compare the bioaccessibility methodologies for As, Cd and Pb (Oomen et al. 2002; Van de Wiele et al. 2007), such comparisons have not been undertaken for organic contaminants.

In vitro assays have the potential to overcome the time and expense limitations of in vivo studies, thereby providing a surrogate measurement of bioavailability that is quick and inexpensive compared to animal models (Basta et al. 2001; Ruby et al. 1996). However, in order to validate the use of in vitro assays as a surrogate measure of contaminant bioavailability, the relationship between in vivo bioavailability and in vitro bioaccessibility needs to be established.

Although data on As bioavailability are accumulating in the literature, only a few studies have correlated arsenic bioavailability with As bioaccessibility as determined by in vitro assays. In the study of Rodriguez et al. (1999), there was no statistical difference in the relative availability of As in mining and smelting material when measured by in vitro (IVG method) or in vivo (swine feeding trials) methods. However, the calcine samples analysed using in vitro methods were not statistically equivalent to the in vivo method as As bioavailability was underestimated by the in vitro method. In the studies of Juhasz et al. (2007b, 2008), the authors found a significant correlation between As bioaccessibility as measured by the simplified bioaccessibility extraction test (SBET) method (also known as the SBRC gastric phase or RBALP) and As relative bioavailability as measured by the in vivo method (Pearson's correlation =0.92, n=49); this supports that in vitro assessment of contaminated soils can provide a good prediction of in vivo As relative bioavailability using Eq. 2.

$$\text{Invivo As bioavailability}\left(\text{mg kg}^{-1}\right) = 14.19 + 0.93 \times \left[\text{SBET As bioaccessibility}\left(\text{mg kg}^{-1}\right)\right] \quad (2)$$

Whilst some variability was observed in the data collected during in vivo studies, this may be attributable to physiological intraspecies variability including genetic factors, disparity in stomach clearance times, stomach pH and the rates of arsenic absorption. The same variability was not evident in the in vitro assay with the data being extremely reproducible under laboratory conditions.

In a recent study by Juhasz et al. (2009a, b), As bioaccessibility in contaminated soils (n=12) was assessed using four in vitro assays (SBRC, IVG, PBET, DIN). In vitro results were compared to in vivo As relative bioavailability data (swine assay) to ascertain which methodologies best correlate with the in vivo data. Arsenic bioaccessibility in contaminated soils varied depending on the in vitro method employed. For the SBRC and IVG methods, As bioaccessibility generally decreased when gastric phase values were compared to the intestinal phase. In contrast, extending PBET and DIN assays from the gastric to the intestinal phase resulted in an increase in arsenic bioaccessibility for some soils tested. A meta-analysis of the in vitro and in vivo results demonstrated that the in vitro assay encompassing the SBRC gastric phase provided the best prediction of in vivo As relative bioavailability (Pearson's correlation=0.87; Juhasz et al. 2011). However, As relative bioavailability could also be predicted using the gastric or intestinal phases of IVG, PBET and DIN assays, but with varying degrees of confidence (r^2=0.53–0.67, Pearson's correlation=0.73–0.82; Juhasz et al. 2009a, b).

Research undertaken as part of a USEPA study (Drexler and Brattin 2007; USEPA 2007b) determined that the dissolution of Pb phases following gastric phase extraction provided a good prediction of Pb relative bioavailability determined using juvenile swine. Similar results were obtained by Ruby et al. (1996) (PBET method and a Sprague–Dawley rat model), Denys et al. (2012) (UBM method and a swine model) and Schroder et al. (2004) (IVG method and a swine model). Poor in vivo–in vitro correlations were obtained for intestinal phase data and Pb relative bioavailability, which was attributed to the complex non-equilibrium chemical system for Pb in the small intestines (Ruby et al. 1996). It was suggested, however, that the use of the small intestinal phase data would be preferable as a measure of Pb bioaccessibility (Ruby et al. 1996).

Whilst a good relationship was observed between gastric phase Pb dissolution and in vivo Pb relative bioavailability, the USEPA (2007a) cautioned that the majority of samples

Table 9 Assessment of arsenic bioaccessibility using in vitro gastrointestinal extraction methods

As source	As (mg kg^{-1})	In vitro method	As bioaccessibility	Reference
Herbicide (18)	22–1,345	SBRC gastric	6-89 %	Juhasz et al. (2007a)
Pesticide (13)	39–3,034		9–89 %	Diacomanolis et al. (2007)
Mine waste (4 composite waste types from 60 samples)	180±50–1,340±720	PBET	3.9±0.5–8.5±4.35 %	
Mine waste (9)	210–2,570	PBET	7.1±1.7–10.2±2.1 %	Bruce (2004)
Mine waste (8)	606–1,2781			
Geogenic (11)	13–422		5–35 % 1–22 %	Smith et al. (2009)
Industrial (2)	55–236	SBET DIN (fasted)	11.50 % 18.44 %	Oomen et al. (2002)
		DIN (fed)	11.30 %	
		BARGE	19.95 %	
		SHIME	1.6 %	
		TIM	15.52 %	
Mine waste (15)	233–17,500	IVG gastric IVG intestinal	3.6–24.8 % 3.5–22.7 %	Rodriguez et al. (1999)
		PBET gastric	1.4–18.3 %	
		PBET intestinal	1.5–12.5 %	
CCA (20)	37.4–310	IVG gastric IVG intestinal	15.8–63.6 % 17.0–66.3 %	Girouard and Zagury (2009)
CCA (12)	23-220	IVG gastric IVG intestinal	20.7–63.6 % 25.0–66.3 %	Pouschat and Zagury (2006)
Ironstone formations (15)	19–102	PBET	1–10 %	Wragg et al. (2007)
Pesticide (12)	31.3–2,143	IVG gastric IVG intestinal	2–76 % 3–90 %	Sarkar et al. (2007)
Mine waste (87)	249–68,900	PBET	0.5–42 %	Palumbo-Roe and Klinck (2007)
Mineralised soil (20)	123–205		6.8–16.7 %	
Tailings (22)	1,280–204,500		0.6–61.1 %	
Mine waste (3)	1,406–20,000	PBET gastric PBET intestinal	10–12.5 % 16–35.6 %	Williams et al. (1998)
Residential (2)	170–3,900	PBET gastric	34–55 %	Ruby et al. (1996)
House dust (1)		PBET intestinal	31–50 %	
River sediment (9)	33–264	PBET gastric	1–11 %	Devesa-Rey et al. (2008)
Mine waste/soil (27)	204–9,025	BARGE Saliva–gastric–intestinal	10–34 %	Button et al. (2009)
Mine waste (18)	40–824	SBRC gastric SBRC intestinal	0.1–25 % 0–3 %	Navarro et al. (2006)
Residential soil near smelter (10)	214–5,214	PBET intestinal	39–66 %	Carrizales et al. (2006)

used were derived from similar sources (mining and milling activities) and that some forms of Pb that were absent in these soils may not follow the observed correlation (USEPA 2007b). This was highlighted in the study of Marschner et al. (2006), who reported that the absolute and relative bioavailability of Pb in five urban and industrial soils, determined using liver, kidney, bone and urine data from soil dosed minipigs, was not related to Pb bioaccessibility determined using the standardised German in vitro assay (Hack and Selenka 1996).

In the study of Juhasz et al. (2009a b), Pb relative bioavailability in contaminated soils was determined using an in vivo swine assay, whilst Pb bioaccessibility was assessed using an in vitro method (SBRC) encompassing the gastric (SBRC-G) and intestinal (SBRC-I) phases. Initially, bioaccessibility studies were performed with a Pb reference material (Pb acetate, 1–10 mg l^{-1}) in order to determine the influence of pH on Pb solubility. In the gastric phase (pH 1.5), Pb solubility was 100 % (100±2.9 %, n=16) irrespective of the Pb concentration added; however, when

Table 10 Assessment of lead bioaccessibility using in vitro gastrointestinal extraction methods

Pb source	Pb (mg kg^{-1})	In vitro method	Pb bioaccessibility	Reference
Urban soils (15)	32–6,330	DIN	2–21 % (without milk powder, 11–56 % (with milk powder)	Marschner et al. (2006)
Mine waste (4)	1,030–5,820	PBET gastric PBET intestinal	0.5–6 % 0.1–0.7 %	Ruby et al. (1993)
Mine waste (6)	140–1,800	SBET	65–110 %	Schaider et al. (2007)
Mine waste (18)	1,270–14,200	IVG gastric IVG intestinal	0.7–36.3 % 0.02–1.16 %	Schroder et al. (2004)
Mine waste (7)	1,388–10,230	PBET gastric PBET intestinal	1.3–83 % 2.7–54 %	Ruby et al. (1996)
Carpet dust (15)	209–1,770	PBET gastric PBET intestinal	52–77 % 5–32 %	Yu et al. (2006)
Mine waste (1) Urban soil (1) Residential soil (1)	68–2,924	Saliva, gastric and intestinal phases	39–69 %	Hamel et al. (1998)
Residential soil (2) Mine waste (2)	2,141–77,007	BARGE Saliva–gastric–intestinal	15-56 % 5–25 %	Denys et al. (2007)
Mine waste (1)	3,060	PBET DIN BARGE SHIME TIM	13 % (fasted), 22 % (fed) 14 % (fasted), 29 % (fed) 32 % (fasted), 24 % (fed) 2 % (fasted), 24 % (fed) 33 % (fasted), 7 % (fed)	Van de Wiele et al. (2007)
Mine waste (18)	153–4,817	SBRC gastric SBRC intestinal	0.5–86 % 0–80 %	Navarro et al. (2006)
Residential soil near smelter (10)	391–4,062	PBET intestinal	13–64 %	Carrizales et al. (2006)
Soil contaminated with pottery flakes (10)	50–2,400	BARGE	28–73 %	Oomen et al. (2003)
Urban soil (2) Incinerator site (3)	646–3,905	SBRC gastric SBRC intestinal Rel-SRBC intestinal	36–64 % 1.2–2.7 % 14–26 %	Juhasz et al. (2009a, b)
Industrial (2)	612–6,380	SBET DIN (unfed) DIN (fed) BARGE SHIME TIM	56 %, 69% 23 %, 40 % 16 %, 31 % 29 %, 66 % 1 %, 4 % 4 %, 13 %	Oomen et al. (2002)
Mine waste (4 composite waste types from 60 samples)	550±80–5,450 ±2,700	PBET	10.2±1.5–13.4±2.6 %	Diacomanolis et al. (2007)
Mine waste (9)	170–12,100	PBET	3.7±1.7–24.2±4.8 %	Bruce (2004)

Correlation between in vivo bioavailability and in vitro bioaccessibility

the pH of the intestinal phase was increased to near neutral, Pb solubility decreased to 14.3±7.2 %. In contaminated soils, Pb bioaccessibility varied from 35.7 to 64.1 % and from 1.2 to 2.7 % for the SBRC-G and SBRC-I phases, respectively. When relative bioaccessibility (Rel-SBRC-I) was calculated by adjusting the dissolution of Pb from contaminated soils by the solubility of Pb acetate at pH 6.5 (intestinal phase pH), Rel-SBRC-I values ranged from 11.7 to 26.1 %. The relationship between Pb bioaccessibility determined using either the SBRC-G, SBRC-I or Rel-SBRC-I methods and Pb relative bioavailability, determined using the in vivo swine assay, was ascertained. When Pb relative bioavailability was plotted against Pb bioaccessibility, three distinct data groupings were evident. A comparison of in vitro and in vivo results indicated that the correlation between Pb bioaccessibility and Pb relative bioavailability varied depending on the in vitro methodology used.

Regression models (SPSS, Release 15.0.1, 2006) determined that Rel-SBRC-I provided the best estimate of in vivo Pb relative bioavailability for the soils used in this study. Although research undertaken as part of a USEPA study (Drexler and Brattin 2007; USEPA 2007b)

determined that gastric phase extraction provided a good estimate of Pb relative bioavailability, the relationship between SBRC-G and in vivo Pb relative bioavailability was poor. However, if in vitro–in vivo relationships were calculated using bioaccessibility/bioavailability data expressed as milligrams of available Pb per kilogram, SBRC-G provided a good estimate of Pb relative bioavailability. This was not surprising as the amount of Pb in the intestinal phase is dependent on gastric phase dissolution. A poor relationship was observed between in vivo relative Pb bioavailability and SBRC-I values, as previously reported by Ruby et al. (1996), Schroder et al. (2004) and Marschner et al. (2006).

Knowledge gaps

There is a general lack of acceptance of the use of bioaccessibility data to predict contaminant bioavailability. Several major reasons have been identified for this approach, as stated in "National and international approaches" above.

One of the major limitations for addressing these concerns is the actual cost of any in vivo animal study. Lack of validation between in vitro and in vitro considerably inhibits the regulatory acceptance of any in vitro methodology. Only As and Pb bioavailability in contaminated soil has been reported extensively. However, no single methodology has been used consistently across a number of different inorganic contaminants. A comparison of the commonly employed in vitro bioaccessibility methodologies for inorganic soil contaminants is severely lacking and needs urgent addressing. Furthermore, inclusion of standard reference soils needs to be included in experimental design by researchers to enable the comparison of laboratory efficacy in conducting research. The authors suggest standard reference soils NIST SRM 2711 or NIST SRM 2710, or, at the very least, a well-characterized in-house soil material as part of the QA/QC protocol.

Although it is commonly assumed that a single bioaccessibility methodology may be used to predict the relative bioavailability for a range of contaminants, there is little scientific evidence to support this view. Considerable research needs to be focused on identifying a suitable method that may be used effectively as a surrogate measure for contaminant relative bioavailability.

Conclusions

When assessing the impact of an ingested chemical on human health risk assessment, the chemical's toxicity is influenced by the degree to which it is absorbed from the gastrointestinal tract into the body (i.e. its bioavailability). As oral references doses and cancer slope factors are generally expressed in terms of ingested dose, rather than the absorbed dose, the variability in absorption between different exposure media, chemical forms, etc., may significantly influence risk calculations. In Australia, NEPM HILs (and indeed guideline values from other countries) are derived using a conservative bioavailability default value of 100 %. However, the assumption that 100 % of the soil-borne contaminant is bioavailable may overestimate exposure, thereby influencing risk calculations. As a result, assessment of contaminant bioavailability may help refine exposure modelling for tier 2 human health risk assessment.

In the absence of human studies or the availability of suitable epidemiological data, the relative bioavailability of soil-borne contaminants may be assessed using in vivo methods. Bioavailability assessment using an in vivo model is considered to be the most reliable method for refining exposure models for tier 2 human health risk assessment. Whilst a variety of animal models have been utilised for the assessment of RBA, standard operating procedures for these in vivo models are currently unavailable. However, the USEPA has developed a guidance document for evaluating the bioavailability of metals in soil for use in human health risk assessment (USEPA 2007a), whilst Rees et al. (2009) detailed an in vivo swine assay for the determination of relative arsenic bioavailability in contaminated soil and plant matrices. The use of juvenile swine for the assessment of RBA is prescribed by the USEPA; however, other in vivo models (e.g. rodents, primates) may be utilised if deemed suitable for the contaminant of interest. Bioavailability endpoints may include the determination of inorganic contaminants in blood, organs, urine and faeces, urinary metabolites, DNA adducts and enzyme induction (e.g. cytochrome P450 monooxygenases).

Several in vitro methods have been developed for the prediction of contaminant relative bioavailability. These in vitro methodologies do not attempt to replicate the conditions found in vivo, but mimic key processes such as contaminant dissolution. In vitro assays have the potential to overcome the time and expense limitations of in vivo studies, thereby providing a surrogate measurement of bioavailability that is quick and inexpensive compared to animal models. However, in order for an in vitro bioaccessibility test system to be useful in predicting the in vivo relative bioavailability of a test material, it is necessary to establish empirically that a strong correlation exists between the in vivo and in vitro results across a variety of sample types.

A limited number of studies have established the relationship between in vivo RBA and in vitro bioaccessibility. For inorganic contaminants, these studies have been limited to As (Basta et al. 2007; Juhasz et al. 2007a, b, 2009a, b; Rodriguez et al. 1999) and Pb (Drexler and Brattin 2007; Juhasz et al. 2009a, b; Schroder et al. 2004; USEPA 2007b) and more recently extended to Cd (Schroder et al. 2003;

Juhasz et al. 2010), in addition to As and Pb with the UBM test (Denys et al. 2012), with only the USEPA (2007b) study gaining regulatory acceptance. Data are emerging for other inorganic contaminants (e.g. Cd, Ni); however, the correlation between RBA and bioaccessibility is lacking or limited information is available, which precludes confidence in the determination of in vivo–in vitro relationships.

Figure 2 illustrates when and how bioavailability and bioaccessibility can be incorporated into the risk assessment framework for the incidental soil ingestion exposure pathway. The recommended decision framework is intended for the collection of data to inform site-specific risk-based decisions. The decision framework does not support the use of invalidated models for site-specific risk assessment. Within the decision tree, the cost associated with obtaining more reliable bioavailability data as opposed to bioaccessibility as a surrogate measure of bioavailability will depend on individual circumstances including the land value, level of contamination and its remediation goal.

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